Advanced Glycation End Products Are Associated With Pulse Pressure in Type 1 Diabetes

The EURODIAB Prospective Complications Study

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Abstract—We investigated the associations of pulse pressure (a measure of arterial stiffness) with the early glycation products hemoglobin A1c (HbA1c) and Amadori albumin and the advanced glycation end products pentosidine, N^ϵ-(carboxymethyl)lysine and N^ϵ-(carboxyethyl)lysine in a large group of type 1 diabetic individuals of the EURODIAB Prospective Complications Study. We did a cross-sectional nested case-control study from the EURODIAB Prospective Complications Study of 543 (278 men) European individuals with type 1 diabetes diagnosed at <36 years of age. We used linear regression analyses to investigate the association of pulse pressure with glycation products. Pulse pressure was significantly associated with plasma levels of N^ϵ-(carboxymethyl)lysine and N^ϵ-(carboxyethyl)lysine but not with HbA1c, Amadori albumin, and urinary levels of pentosidine. Regression coefficients adjusted for age, sex, mean arterial pressure, and duration of diabetes were 0.09 mm Hg ($P=0.003$) per 1 μM/M lysine N^ϵ-(carboxymethyl)lysine; 0.24 mm Hg ($P=0.001$) and -0.03 mm Hg ($P=0.62$) per 1 μM/M lysine N^ϵ-(carboxyethyl)lysine (in individuals with and without complications, respectively; P interaction$=0.002$); and 0.50 mm Hg ($P=0.16$) per 1% HbA1c; 0.07 mm Hg ($P=0.12$) per 1 U/mL Amadori albumin; and 0.77 mm Hg ($P=0.48$) per 1 nmol/mmol creatinine pentosidine. In young type 1 diabetic individuals, arterial stiffness is strongly associated with the advanced glycation end products N^ϵ-(carboxymethyl)lysine and N^ϵ-(carboxyethyl)lysine. These findings suggest that the formation of advanced glycation end products is an important pathway in the development of arterial stiffness in young type 1 diabetic individuals. (*Hypertension*. 2005;46:232-237.)

Key Words: diabetes mellitus ■ arteriosclerosis ■ elasticity ■ aging

Increased arterial stiffness is associated with increased cardiovascular risk[1] because it increases myocardial afterload and oxygen demand, leads to left ventricular hypertrophy, and limits coronary filling during diastole.[2] Recent studies have shown that arterial aging in type 1 and type 2 diabetes is accelerated.[3,4] However, the exact mechanisms that are involved in accelerated stiffening of arteries remain unknown. One of the main mechanisms thought to be involved is the formation of advanced glycation end products (AGEs), especially in diabetic individuals. AGEs that form on the arterial wall can cause cross-linking of collagen molecules, which may lead to loss of collagen elasticity and a subsequent reduction in arterial elasticity.[5] Recently, cross-link breakers have been demonstrated to decrease arterial stiffness in humans, providing additional evidence for the importance of AGE formation in arterial stiffening.[6]

Studies on arterial stiffness have been performed in elderly or type 2 diabetic individuals because arterial stiffness, specifically pulse pressure, tends to increase at >50 years of age.[7,8] However, in type 1 diabetes, pulse pressure increases even at an earlier age.[3] In contrast to mean arterial pressure, which represents the steady component of the propagation of the pressure wave through the arterial system, pulse pressure represents the pulsatile component of circulatory stress. Arterial pulse pressure is determined by cardiac output and arterial stiffness.[9] Variability in pulse pressure is thought to be caused, to a large extent, by variability in arterial stiffness.

The influence of AGE formation on arterial stiffness in young type 1 diabetic individuals has not been investigated previously. Therefore, we investigated the associations of the early glycation products hemoglobin A1c (HbA1c) and Amadori albumin and the AGEs pentosidine, N^ϵ-(carboxymethyl)lysine (CML), and N^ϵ-(carboxyethyl)lysine (CEL) with pulse pressure as a measure of arterial stiffness in a large group of type 1 diabetic individuals of the EURODIAB Prospective Complications Study.

Methods

Study Population

Full details of the design, methods, and recruitment in the EURODIAB Prospective Complications Study have been published previ-

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ously.[10] The EURODIAB Prospective Complications Study is a follow-up of the EURODIAB IDDM (insulin-dependent diabetes mellitus) Complications Study.[11] Baseline investigations (1988 to 1991) were performed on 3250 men and women with type 1 diabetes drawn from 31 European centers. All subjects gave informed consent, and the study was approved by local ethics committees. Sample selection was stratified by sex, age group, and duration of diabetes to ensure sufficient representation in all categories. Type 1 diabetes was clinically defined as a diagnosis made at <36 years of age, with a continuous need for insulin therapy within 1 year of diagnosis. The follow-up was performed on average 7 to 9 years later. Of the 3250 patients, 1880 (57.8%) returned for examination.[10,12,13] At follow-up, a cross-sectional nested case-control study on AGEs was performed (n=543). Data presented here were based on these cross-sectional data. AGEs could not be determined at baseline because these samples have run out.

We assessed microvascular complications and cardiovascular disease, did a physical examination, measured height, weight, waist and hip circumference, and resting blood pressure, obtained information on smoking habits, and measured biochemical variables according to a standardized protocol.[11] Albumin excretion rates were measured from 2×24-hour urine collections at a single center as described previously.[10] Microalbuminaria and macroalbuminuria were defined as albumin excretion rates of between 20 and 200 μg per minute and >200 μg per minute, respectively. We estimated glomerular filtration rate (GFR) by the Cockcroft–Gault formula. Retinopathy was assessed from retinal photographs according to the EURODIAB protocol.[14] Cardiovascular disease was defined as a positive medical history of a cardiovascular event, including myocardial infarction, angina, coronary artery bypass graft or stroke, or ischemic changes on a Minnesota-coded ECG.[15]

Blood pressure was measured by a random-zero sphygmomanometer (Hawskley). With an appropriately sized cuff, 2 blood pressure readings were taken from the right arm with the patient in a seated position after resting for 5 minutes. Readings were taken from the top of the meniscus, and measurement was recorded to the nearest 2 mm Hg. Diastolic pressure was recorded at the disappearance of sound (Korotkoff phase V). Data presented here are based on the mean of 2 measurements. Mean arterial pressure was defined as two thirds of diastolic plus one third of systolic pressure and pulse pressure as systolic minus diastolic pressure. Hypertension was defined as a systolic pressure of ≥140 mm Hg, a diastolic pressure of ≥90 mm Hg, or the current use of blood pressure–lowering drugs.

Laboratory Measurements

HbA1c was measured by an enzyme immunoassay using a monoclonal antibody against HbA1c (DAKO; n=535).[16] Amadori albumin was determined in a competitive ELISA as described previously (n=535).[17] Pentosidine levels were determined in unhydrolyzed urine as described previously (n=536).[18] Urinary excretion of pentosidine was normalized for urine concentration by expressing it as nmol pentosidine/mmol urinary creatinine. CML and CEL were determined in plasma as described previously[19] with an interassay coefficient of variation of 6.0% (n=536 and n=535, respectively). CML and CEL were normalized for lysine concentration by expressing it as $\mu M/M$ lysine. Fasting follow-up blood samples were sent to central laboratories for analyses of lipid profile.[15]

Statistical Analysis

Because this study was designed to study determinants of diabetic complications, we used a nested case-control approach to compare individuals with and without complications. Cases were selected to have the greatest complication burden as possible to provide sufficient numbers for subgroup analyses. Controls were selected to be completely free of complications (including neuropathy). Thus, cases were all those with cardiovascular disease or proliferative retinopathy or macroalbuminuria at follow-up and all those with microalbuminuria and some degree of retinopathy (n=348). Controls were all those who had no evidence of cardiovascular disease, retinopathy, or neuropathy, and were normoalbuminuric at follow-up (n=195). Cases and controls were unmatched so that the impact of key

variables, such as age, could still be assessed, and any adjustments were taken care of at the analysis stage.

All analyses were performed with SPSS 11.5 for Windows. We focused on the cross-sectional associations of pulse pressure, systolic pressure, and diastolic pressure with HbA1c, Amadori albumin, pentosidine, CML, and CEL by use of linear regression analyses. We initially performed these analyses stratified by the presence or absence of complications. Then we investigated, by use of interaction terms of glycation products with the presence of complications, whether findings across strata were significantly different. Because findings were similar across strata, except those involving CEL, we combined the groups, except in analyses involving CEL.

P values <0.05 were considered statistically significant, except for interaction terms, where P values <0.10 were considered statistically significant.

Results

Table 1 shows the characteristics of the study population per tertile of pulse pressure. As anticipated, cardiovascular risk factor status was worst in the highest tertile of pulse pressure. However, HbA1c levels did not differ by tertile of pulse pressure. Amadori albumin, CEL, and pentosidine were significantly and positively related to pulse pressure. The association with CML was borderline significant ($P=0.06$).

Table 2 shows the associations of pulse pressure, systolic pressure and diastolic pressure with HbA1c and Amadori albumin. Pulse pressure, systolic pressure, and diastolic pressure levels were not associated with HbA1c. Pulse pressure and systolic pressure were associated with Amadori albumin; however, this association disappeared after adjustments for age, sex, mean arterial pressure, and duration of diabetes (model 1).

Table 3 shows the associations of pulse pressure, systolic pressure, and diastolic pressure with AGEs. Pulse pressure and systolic pressure were associated with pentosidine and CML in crude analyses. In adjusted analyses, the associations with CML remained present, whereas the association with pentosidine disappeared. Diastolic pressure was associated inversely with CML in adjusted analyses. The associations of pulse pressure, systolic pressure, and diastolic pressure with CEL were significantly stronger in individuals with complications compared with those without complications (Table 3; interaction analyses). Therefore, analyses were stratified for complication status. Pulse pressure and systolic pressure were strongly associated with CEL in individuals with complications in crude and adjusted analyses (Table 3). Diastolic pressure was inversely associated with CEL in individuals with complications in adjusted analyses. Measures of blood pressure were not associated with CEL in individuals without complications. The interaction of CEL with complications in the association of pulse pressure with CEL indicates that this association is stronger in the presence of complications.

Additional Adjustments

Additional adjustments for GFR, body mass index, waist-to-hip ratio, lipid profile, HbA1c (when this was not the dependent variable), the presence of retinopathy, microalbuminaria or macroalbuminuria, or cardiovascular disease, and the use of antihypertensive or lipid-lowering drugs did not materially change the results presented in Tables 2 and 3.

TABLE 1.   Characteristics of 543 Type 1 Diabetic Patients per Tertiles of Pulse Pressure

Variable	First Tertile of Pulse Pressure (17–39.5 mm Hg)	Second Tertile of Pulse Pressure (40–51 mm Hg)	Third Tertile of Pulse Pressure (51.5–129 mm Hg)	P Value for Trend
Clinical characteristics				
Sex, men/women	85/95	103/80	90/90	0.2
Age, years	34.9±7.9	38.7±8.8	45.6±10.5	<0.001
Duration of type 1 diabetes, years	15.6 (10.9–21.8)	20.0 (14.4–25.6)	26.7 (19.5–32.9)	<0.001
Body mass index, kg/m^2	23.9±2.8	24.3±3.1	25.3±3.7	<0.001
Waist-to-hip ratio	0.88±0.15	0.89±0.14	0.89±0.13	0.7
Smoking status, never/past/current, %	47/22/31	35/30/35	39/36/25	0.1
Complications				
No/background/proliferative retinopathy, %	59/24/17	49/26/26	24/33/43	<0.001
Normoalbuminuria/microalbuminaria/macroalbuminuria, %	75/13/12	63/16/21	44/17/39	<0.001
GFR, mL/min per 1.73 m^2	110.4±21.8	104.7±23.6	94.3±28.3	<0.001
Cardiovascular disease (n=489), %	19	29	35	<0.001
Blood pressures				
Systolic and diastolic blood pressure, mm Hg	108±11/75±10	120±12/75±11	142±18/76±13	<0.001/0.5
Mean arterial pressure, mm Hg	86±10	90±11	98±13	<0.001
Pulse pressure, mm Hg	33±5	45±4	66±15	—
Hypertension, %	25	29	72	<0.001
Use of antihypertensive drugs, %	17	25	53	<0.001
Lipid profile				
Total cholesterol, mmol/L	5.02±1.10	5.28±1.02	5.61±1.34	<0.001
HDL cholesterol, mmol/L	1.64±0.43	1.64±0.40	1.61±0.47	0.7
LDL cholesterol, mmol/L	2.88±0.89	3.13±0.97	3.36±1.24	<0.001
Triglycerides, mmol/L	0.93 (0.71–1.27)	0.95 (0.74–1.36)	1.14 (0.86–1.68)	<0.001
Glycation products				
HbA1c, %	8.5±1.6	8.4±1.5	8.7±1.7	0.2
Amadori albumin, U/mL	44.8±15.0	44.2±11.1	47.9±13.6	0.02
CML, μM/M lysine	55±15	56±17	59±21	0.06
CEL, μM/M lysine	29±11	30±10	33±13	0.01
Pentosidine, nmol/mmol creatinine	0.44 (0.32–0.60)	0.44 (0.32–0.64)	0.48 (0.35–0.71)	0.04

Data are presented as No., percentage, or mean±SD, except for duration, triglycerides, and pentosidine, which are shown as median (interquartile range).

Discussion

The main outcome of this study is that pulse pressure, an estimate of arterial stiffness, was strongly and independently associated with the AGEs, CML, and CEL in young type 1 diabetic individuals. This is the first study to demonstrate that AGEs are associated with arterial stiffness in relatively young type 1 diabetic individuals. This interpretation is strengthened by the finding that CML and CEL were directly associated with systolic pressure and inversely with diastolic pressure, because greater arterial stiffness will increase systolic and decrease diastolic pressure. In addition, these associations were independent of mean arterial pressure (ie, the static blood pressure factor).

The association of pulse pressure with CEL was significantly stronger in type 1 diabetic individuals with complications compared with individuals without complications, which may indicate that type 1 diabetic individuals with complications may be especially vulnerable to the arterial stiffness–increasing effects of CEL. In contrast, pulse pres-

sure was not independently associated with the early glycation products HbA1c and Amadori albumin and the AGE pentosidine.

CML and CEL are noncross-linking AGEs, yet were strongly associated with pulse pressure. AGEs are formed by a nonenzymatic reaction between reducing sugars and proteins. These stable compounds accumulate slowly throughout the life span and are thought to contribute to age- and diabetes-associated structural changes in the cardiovascular system, such as increased vascular stiffness.[5] The mechanisms underlying this AGE-induced effect on vascular stiffness include the formation of collagen cross-linking that alters the structure and function of the extracellular matrix.[5] However, the introduction of CML or CEL moieties on proteins may affect the properties of the modified proteins but does not cause cross-linking in or between proteins.[20,21] Therefore, our findings strongly suggest that the effect of CML and CEL on arterial stiffness involves other mechanisms than cross-linking, such as ligation of the receptor for

TABLE 2.   Cross-Sectional Associations of Pulse Pressure, Systolic Pressure, and Diastolic Pressure With HbA1c and Amadori Albumin

Early Glycation Products	Pulse Pressure, mm Hg			Systolic Pressure, mm Hg			Diastolic Pressure, mm Hg		
	β	SE	P Value	β	SE	P Value	β	SE	P Value
HbA1c, %									
Crude	0.85	0.45	0.06	0.96	0.54	0.08	0.11	0.31	0.73
Model 1	0.50	0.36	0.16	0.33	0.24	0.16	−0.17	0.12	0.16
Amadori albumin, U/mL									
Crude	0.16	0.05	0.003	0.14	0.07	0.03	−0.02	0.04	0.66
Model 1	0.07	0.04	0.12	0.05	0.03	0.12	−0.02	0.01	0.12

Model 1 was adjusted for age, sex, mean arterial pressure, and duration of diabetes. A regression coefficient (β) of 0.85 (top left) indicates that per 1% increase in HbA1c, pulse pressure increases with 0.85 mm Hg.

SE indicates SE of the regression coefficient.

AGE (RAGE). RAGE is highly expressed in the endothelium of activated vessels.[22] Recent findings demonstrated that CML is a ligand for RAGE.[23] Binding of circulating CML-modified proteins to RAGE leads to activation of extracellular-regulated kinase 1/2 (ERK1/2), activation of nuclear factor κB, secretion of proinflammatory cytokines, and modulation of gene expression in several cell types, such as monocytes, endothelial cells, and vascular smooth muscle cells.[24] This may lead to changes in the production of extracellular matrix proteins, such as collagen and elastin structure, and alterations in vascular elasticity.

In contrast to CML and CEL, the cross-linking AGE pentosidine was not associated with pulse pressure. Although the lack of an association of pulse pressure with pentosidine may suggest that cross-linking AGEs are not important in the development of increased arterial stiffness, the findings that AGE–cross-link breakers, such as ALT-711, are able to decrease arterial and myocardial stiffness[5,6] suggests that cross-linking by AGEs is important in this process. We do not have a clear explanation why we did not find such an association in this study. However, we measured pentosidine in its free form in urine, which may be a poor reflection of AGE-induced cross-linking in the vascular wall.

We cannot establish whether CML and CEL are involved in arterial stiffness or rather serve as markers of the process of AGE formation. However, the finding that the AGE pentosidine was not associated with pulse pressure argues against the latter idea. Because CML and CEL can be formed by glycoxidation and by oxidation alone, whereas pentosidine is a specific marker of glycoxidation,[25,26] the association of arterial stiffness with CML and CEL may reflect the involvement of increased oxidative stress in arterial stiffening, whereas glycoxidation, per se, may be less important.

In contrast to CML, which can be formed by multiple pathways such as via glyoxal, myeloperoxidase, or the peroxidation pathway, the primary pathway of CEL formation is during the reaction of methylglyoxal with lysine residues. Therefore, CEL is a specific indicator of methylglyoxal, whereas CML is not. Methylglyoxal has been demonstrated to be the most important precursor in the formation of AGEs.[27] The interaction of CEL with complications in the association between arterial stiffness and CEL may be explained by an increase in methylglyoxal in the presence of diabetic complications. Indeed, increased concentration of methylglyoxal has been linked directly to vascular complications,[28] although the mechanisms and the particular methylglyoxal adducts involved herein are unknown. Whether methylglyoxal-modified proteins are also ligands for AGE receptors is unknown, although the effects of high concentrations of methylglyoxal on cultured human endothelial cells on tyrosine phosphorylation, the phosporylation of ERK1/2, c-JUN, and p38 mitogen-activated protein kinase, and the induction of apoptosis[29] suggest the involvement of a cellular receptor.

Pulse pressure was not associated with the early glycation products HbA1c and Amadori albumin. These early glycation products are highly reversible and are therefore thought to be relatively unimportant with respect to arterial stiffening.

Although we found a significant association of pulse pressure with CML and CEL, we cannot establish that these AGEs play a causal role in the development of increased arterial stiffness because of the cross-sectional setting of this study. However, a large body of evidence supports an important role for AGEs in the pathogenesis of diabetes-related vascular damage. In addition, CML depositions were specifically found in the arterial wall.[30] The finding that collagen cross-link breakers can improve arterial compliance provides additional evidence for the importance of AGE cross-linking in arterial stiffening.[6]

In summary, this study demonstrates an association of arterial stiffness with the AGEs CML and CEL but not with Hba1c, Amadori albumin, and pentosidine. These results suggest that AGEs are an important pathway in the development of arterial stiffness even in quite young type 1 diabetic individuals.

Perspectives

We have shown previously that pulse pressure shows a steep increase with age in young individuals with type 1 diabetes (mean age 33 years), that this association is stronger in individuals with (micro)albuminuria than in those with normoalbuminuria, and that pulse pressure is positively associated with risk of incident cardiovascular disease.[3] Our present data suggest that the formation of AGEs is an important pathway in the development of arterial stiffness in young type 1 diabetic individuals. A crucial next step is to investigate whether AGEs are causally related to arterial stiffness and

TABLE 3.   Cross-Sectional Associations of Pulse Pressure, Systolic Pressure and Diastolic Pressure With Pentosidine, CML, and CEL

Advanced Glycation Products	Pulse Pressure, mm Hg			Systolic Pressure, mm Hg			Diastolic Pressure, mm Hg		
	β	SE	P Value	β	SE	P Value	β	SE	P Value
Pentosidine, nmol/mmol creatinine									
Crude	5.37	1.32	<0.001	4.77	1.61	0.001	−0.60	0.92	0.52
Model 1	0.77	1.11	0.48	0.52	0.74	0.48	−0.26	0.37	0.48
CML, μM/M lysine									
Crude	0.16	0.04	<0.001	0.19	0.05	<0.001	0.03	0.03	0.26
Model 1	0.09	0.03	0.003	0.06	0.02	0.003	−0.03	0.01	0.003
CEL, μM/M lysine									
Individuals with complications									
Crude	0.48	0.08	<0.001	0.59	0.10	<0.001	0.11	0.06	0.056
Model 1	0.24	0.07	0.001	0.16	0.05	0.001	−0.08	0.02	0.001
Individuals without complications									
Crude	0.01	0.07	0.87	0.07	0.09	0.44	0.06	0.07	0.42
Model 1	−0.03	0.06	0.62	−0.02	0.04	0.62	0.01	0.02	0.62
Interaction analyses adjusted for model 1									
Intercept	−15.19	5.54	0.006	−10.13	3.70	0.01	5.06	1.85	0.01
CEL, per μM/M lysine	−0.05	0.08	0.571	−0.03	0.06	0.57	0.02	0.03	0.57
Age, per year	0.37	0.07	<0.001	0.25	0.05	<0.001	−0.12	0.03	<0.001
Sex, male 1, female 2	1.68	1.13	0.138	1.12	0.75	0.14	−0.56	0.38	0.14
Mean arterial pressure, per mm Hg	0.41	0.05	<0.001	1.27	0.03	<0.001	0.87	0.02	<0.001
Duration of diabetes, per year	0.43	0.09	<0.001	0.29	0.06	<0.001	−0.15	0.03	<0.001
Presence of complications, no 0, yes 1	−7.51	3.46	0.030	−5.01	2.31	0.03	2.50	1.15	0.03
CEL×presence of complications	0.32	0.11	0.002	0.21	0.07	0.002	−0.11	0.04	0.002

Model 1 was adjusted for age, sex, mean arterial pressure, and duration of diabetes. A regression coefficient (β) of 5.37 (top left) indicates that per 1 nmol/mmol creatinine increase in pentosidine pulse pressure increases with 5.37 mm Hg.

SE indicates SE of the regression coefficient.

Pentosidine was ln-transformed due to a skewed distribution.

The complete interaction model can be used to calculate the expected change in pulse pressure when CEL increases. For example, in a man aged 35 years with a mean arterial pressure of 90 mm Hg, diabetes duration of 20 years, with complications, pulse pressure is expected to increase by 2.7 mm Hg per 10 μM/M lysine increase in CEL, whereas in a similar person without complications, pulse pressure is expected to change with −0.5 mm Hg (calculated as pulse pressure=−15.19+(−0.05×CEL)+(0.37×age)+(1.68×sex)+(0.41×mean arterial pressure)+(0.43×duration of diabetes)+(−7.51×presence of complications)+(0.32×CEL×presence of complications). Note that the effect of CEL and complications on systolic pressure and diastolic pressure can be calculated in a manner analogous to that shown for pulse pressure.

cardiovascular disease in a longitudinal setting. If so, AGE-breaking agents may be used in clinical practice for cardiovascular risk reduction in type 1 diabetes.

Appendix

EURODIAB Prospective Complications Study Group

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